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Isolation and Characterization of Mutations Affecting the Transport of Hexose Phosphates in *Escherichia coli*

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Mutants of *Escherichia coli* defective in the hexose phosphate transport system were isolated. Negative selection by penicillin treatment or positive selection with phosphonomycin was employed. These mutants grew normally on all carbon sources other than hexose phosphates. The map location of the mutations in 18 independently isolated mutant strains was investigated by transduction crosses. All of the mutations were found to lie in the same region of the chromosome, in the region represented by min 72 on the Taylor map. The order of the genes in this region was found to be *mtl-cysE-pyrE-uhp-bgl-ilv*. Revertants of some of the mutants exhibited altered regulatory control of this transport system.

The ability of *Escherichia coli* to utilize hexose phosphates as carbon source is dependent on the activity of an inducible transport system (5, 12, 16). This transport system allows the active accumulation of a number of hexose phosphates, apparently without alteration of the transported molecules. The energy for this process can be derived from electron transport through various membrane-bound dehydrogenases (3; Lombardi and Kaback, *personal communication*). The transport system is induced by glucose-6-phosphate (G6P) in the culture medium, but not by the intracellular pool of G6P (7, 17, 18). The induction process is specific for exogenous G6P, whereas the transport process exhibits a much lower degree of substrate specificity. This fact suggests the presence of a regulatory molecule at the cell surface which is distinct from the transport system.

This communication represents an initial report on a genetic characterization of the system for the uptake of hexose phosphates. Reports from Kornberg's laboratory described mutants which were unable to transport G6P and also mutants in which this transport system was expressed constitutively (4, 9). Both types of mutations were cotransduced with *pyrE* (located at min 72 on the Taylor map [15]), but their orientation relative to *pyrE* was not established. Kornberg et al. proposed the genetic designation *uhp*, for uptake of hexose phosphates. We report here the application of a number of other selection procedures for the isolation of mutants affecting this transport system. A number of these mutants are characterized with respect to their map location and the properties of revertants derived from them.

MATERIALS AND METHODS

Media. The buffer for growth of cells was medium A of Davis and Mingioli (2), supplemented with appropriate growth factors, such as thiamine (1 μg/ml), amino acids (each, 100 μg/ml), and uracil (40 μg/ml). Carbohydrates were added to a final concentration of 0.5%, with the exceptions of G6P or salicin, whose concentrations were 0.2%. Fermentation of sugars was scored on tetrazolium indicator plates. Media were solidified with 2% agar. Medium LB was employed in the genetic crosses (11). Phosphonomycin was a gift from Merck Chemical Division, and freshly prepared solutions were used at a final concentration of 10 μg/ml.

Strains. The *E. coli* strains used in this work are listed in Table 1. Strain RK 1041 was constructed from W1450, obtained from B. Low. The *pyrE60* mutation was introduced from RK1032 (AB4518 bgl^+ by cotransduction with bgl^+. The *cysE* mutation was introduced by exposure to a P1 lysate propagated on JM 70, followed by one round of penicillin selection for cysteine auxotrophs. The bgl^+ marker was isolated and scored by selection for growth on salicin as carbon source, since the utilization of salicin (a β-glucoside) requires the proper functioning of the *bgl* system (13).

TABLE 1. *Escherichia coli strains*

Strain	Mating type	Pertinent markers	Origin
E15	Hfr Cavalli	*thi*	E. Lin
Lin 6	Hfr Cavalli	*thi glpT*	E. Lin
RK21	Hfr (JC 12)	*thi purC metB pyr-E60 strA*	
RK22	Hfr (KL 25)	*thi pyrE60*	
W1450	F^-	*ilv argH his metB mtl*	B. Low
AB4518	F^-	*thr leu argE3 proA2 his pyrE60*	S. Schaefler (13)
RK1032	F^-	AB4518 *bgl*$^+$	
RK1034	F^-	*ilv argH his metB pyrE60 bgl*$^+$ *mtl*	
JM70	F^-	*thr leu arg his trp cysE*	M. Jones-Mortimer (8)
RK1041	F^-	*ilv argH his metB pyrE60 cysE bgl*$^+$ *mtl*	

Isolation of mutants. When employed, mutagenesis with ethyl methane sulfonate (EMS) was performed by treatment of stationary-phase cultures with 0.04% EMS for 30 min at room temperature. Cells were subjected to penicillin selection with 0.2% G6P as carbon source, and survivors were tested for lack of growth on G6P but normal growth with glucose.

Cultures of strain Lin 6 growing in 1% Casamino Acids supplemented with 0.2% G6P were exposed to phosphonomycin. Incubation was continued for several hours, after which cell lysis was evident. The cells were then washed and plated on glucose medium, and the survivors were tested for their ability to grow on G6P.

Transport assays. The activity of the hexose phosphate transport system was determined by the uptake of labeled G6P. Cells were grown in 1% Casamino Acids supplemented, when appropriate, with 1 mM G6P. When the cells reached mid-log phase, they were harvested, washed once with medium A containing 50 μg of chloramphenicol/ml, and then suspended in this medium to a concentration of 10^9/ml. For determination of transport, 0.9 ml of the cell suspension was added to 0.1 ml of ^{14}C-G6P (5 mM, 1 μCi/ml), and a 0.4-ml sample was filtered after incubation at 25 C for 0.5 and 1.5 min. The filters were washed with 5 ml of medium A and immediately removed from the filtration apparatus. The filters were allowed to dry, and their radioactivity was determined in a scintillation counter.

Transductions. The genetic crosses described in this paper were P1kc-mediated transductions. Phage lysates of donor strains were prepared by adsorbing 10^4 plaque-forming units to 10^7 cells and plating these after 15 min at 37 C in 2.5 ml of LB soft agar (0.8%) on LB plates. All media contained 5 mM $CaCl_2$. After incubation overnight at 37 C, the plates showed almost confluent lysis of the bacteria. The soft-agar layer was scraped into a centrifuge tube, mixed with several drops of chloroform, and centrifuged at 12,000 $\times$ *g* for 10 min. The pellet was washed with 2 ml of LB medium and centrifuged. The combined supernatant fluids usually contained 3 $\times 10^{10}$ to 7 $\times 10^{10}$ plaque-forming units/ml.

The phage lysate was adsorbed to 10^8 recipient cells/ml at a multiplicity of 5 for 30 min at 37 C. The cells were harvested by centrifugation, resuspended in medium A, and plated on selective plates. Selection plates were incubated for several hours at room temperature and then for 36 hr at 37 C. Recombinants were picked to the same selective medium and were then replicated to score the inheritance of the unselected markers.

RESULTS

Isolation of mutants. Mutants of *E. coli* which are unable to grow with glucose-6-phosphate as carbon source, but which grow normally on glucose, could arise from loss of the hexose phosphate transport system. Two selection protocols were used for the isolation of such mutants. The first involved penicillin treatment of EMS-treated cells of strain E15 (wild type) grown with G6P as sole carbon source. Those survivors that grew normally on glucose but with greatly reduced growth rates on G6P were selected. The nine independently isolated mutants designated *uhp* 11 to 15 and 17 to 20 were obtained by this method.

A second, direct selection was based on the fact that phosphonomycin (a bactericidal agent which inhibits the conversion of *N*-acetyl glucosamine to *N*-acetyl muramic acid) enters the bacterial cell on the transport systems for hexose phosphates or α-glycerol-phosphate (1, 6, 10). Strain Lin 6 (*glpT*) was employed as the parental strain so that entry of phosphonomycin on the glycerol-phosphate transport system was eliminated. Cells were grown on Casamino Acids medium supplemented with G6P. The addition of phosphonomycin resulted in extensive cell lysis within 2 hr. After one cycle of phosphonomycin treatment, 2 to 7% of the survivors were defective for growth on G6P; a second cycle of selection yielded cell populations in which 70 to 90% of the survivors were unable to grow on G6P. Nine independent isolates (*uhp* 21 to 29) were obtained by this method.

All 18 mutants chosen for further characterization exhibited wild-type growth rates with glucose as carbon source. Their ability to utilize lactose, gluconate, mannitol, ribose, glycerol, galactose, or Casamino Acids as carbon source appeared to be unimpaired. No significant effect of the addition of cyclic-adenosine monophosphate (5 mM) on the growth of several of these mutants on G6P could be detected. The ability of these mutants to transport ^{14}C-G6P was greatly depressed;

the level of transport in all of these mutants was approximately the same as the level in the uninduced parental strains. Thus, the apparent defect in all of these strains appears to be in either the synthesis or activity of the hexose phosphate transport system.

Genetic characterization. A genetic characterization of these mutants was initiated to determine the number of genetic loci involved in this transport system. Kornberg had shown that mutants with similar properties, although obtained by a different selection procedure, mapped near *pyrE* (9). Conjugation analysis with several of our mutants indicated a similar location.

Phage lysates propagated on each of the 18 Uhp^- strains were employed to transduce three different recipient strains, each carrying the same *pyrE60* mutation. The $pyrE^+$ recombinants obtained were scored for the inheritance of the donor *uhp* allele (Table 2). Each mutation gave approximately 40% cotransduction with *pyrE*.

Unequivocal verification of the close linkage of all of these mutations was provided by the determination of the gene order in this region of the chromosome. For this purpose, strain RK1041 was constructed carrying, in addition to the *mtl*, *pyrE60*, bgl^+, and *ilv* markers, a mutation in the gene *cysE*. This latter marker had been shown to cotransduce with *pyrE*, but its exact location had not been described (8).

This strain was employed as a recipient for P1 phage grown on each of the 18 Uhp^- strains. Selection was made for Cys^+, Pyr^+, or Ilv^+ transductants, and the inheritance of unselected markers was determined by replica-plating. The only marker showing linkage to *ilv* was *bgl*, which gave 31% cotransduction (93 of 300 ilv^+ recombinants). The distribution of donor markers among Cys^+ and Pyr^+ recombinants is presented in Table 3 and Fig. 1. All of the data obtained with each of the 18 phage lysates have been pooled because they all gave essentially identical distributions of inheritance of the unselected markers.

The data in Table 3 indicate that *cysE* is between *mtl* and *pyrE* and that *uhp* is on the opposite side of *pyrE*. Support for these conclusions comes from the distribution of the various recombinant classes, as portrayed in Fig. 1. The gene order is determined from the minority recombinant class, whose formation apparently requires four crossover events. All of these results are consistent only with the gene order *mtl-cysE-pyrE-uhp*.

A similar analysis indicated that *bgl* is located to the right of *uhp*, although *bgl* gave only 2 to 4% cotransduction with *pyrE*. This is consistent with the finding that *bgl* gave 31% cotransduction with *ilv*, whereas no cotransduction of *uhp* with *ilv* was detected. The cotransduction frequencies for these markers, as determined from the data presented, are shown in Fig. 2.

Properties of uhp^+ revertants. The mapping data presented above indicate that all of these mutations affecting the hexose phosphate transport system are located in the same region of the chromosome. It was of interest to determine whether the mutations affected the structural gene for the transport system or

TABLE 2. *Cotransduction of uhp with pyrE*

Donor *uhp* allele	Recipient			Total	Per cent
	RK21	RK22	RK1034		
11	45/100[a]	39/98	191/488	275/686	40.1
12	105/225	35/114	161/399	301/738	40.8
13	39/100	74/222	204/499	317/821	38.6
14	79/175	48/96	45/125	172/396	43.4
15	80/180	127/317	280/569	487/1066	45.7
17	46/100	99/274	135/294	280/668	41.9
18	31/113	90/295	148/365	269/773	34.8
19	87/200	76/177	188/432	351/809	43.4
20	42/99	19/58	168/377	229/534	42.9
21	33/92	20/64	161/325	214/481	44.5
22	50/95	45/121	136/366	231/582	39.7
23	44/100	128/292	156/340	328/732	44.8
24	14/43	10/23	148/369	172/435	39.5
25	37/98	85/295	133/499	255/892	28.6
26	41/97	33/90	127/338	201/525	38.3
27	35/93	—	149/369	184/462	39.8
28	2/10	—	31/82	33/92	35.9
29	48/131	8/33	150/351	206/515	40.0
Total				4,505/11,207	40.2

[a] Number of uhp^-/number of $PyrE^+$ recombinants.

TABLE 3. *Mapping of uhp by transduction*
Donor: mtl^+-$cysE^+$-$pyrE^+$-uhp^--bgl^--ilv^+
Recipient: *mtl-cysE-pyrE*-uhp^+-bgl^+-*ilv*

Selected marker	Total scored	No. that are				
		mtl^+	cys^+	pyr^+	uhp^-	bgl^-
Pyr^+	2,985	270 (9%)	585 (19%)	—	1,190 (40%)	90 (3%)
Cys^+	1,066	604 (57%)	—	152 (14%)	23 (2%)	—

ORDERING OF mtl - cysE - pyrE

Recombinant	Number
mtl-cys-pyr	(1,066)
+ * +	74 (7%)
- * -	384 (36%)
+ * -	530 (50%)
- * +	78 (7%)

Recombinant	Number
mtl-cys-pyr	(2,985)
+ + *	256 (9%)
- - *	2,386 (80%)
- + *	329 (11%)
+ - *	14 (0.5%)

ORDERING OF cysE - pyrE - uhp

Recombinant	Number
cys-pyr-uhp	(1,066)
* + -	18 (1.7%)
* - +	909 (85%)
* + +	134 (13%)
* - -	5 (0.5%)

Recombinant	Number
cys-pyr-uhp	(2,985)
+ * -	153 (5%)
- * +	1,363 (46%)
+ * +	432 (14%)
- * -	1,037 (35%)

FIG. 1. *Ordering of mtl-cysE-pyrE-uhp by transduction. The distribution of the various classes of recombinants obtained from the transduction described in Table 3 is shown. Inheritance of donor markers is represented by the upper bar; those from the recipient, by the lower bar. The selected marker is represented by the asterisk (*). The crossovers necessary to form the recombinant obtained, as required by the order mtl-cysE-pyrE-uhp, are indicated by the vertical lines. A total of 2,985 Pyr⁺ and 1,066 Cys⁺ recombinants were scored in these crosses.*

altered the regulatory system to prevent induction. It has thus far been impossible to detect the presence of a nonfunctional transport system. Hence, the reversion properties of these mutants were investigated on the assumption that revertants of structural gene mutations should exhibit wild-type inducibility. On the other hand, some revertants of a regulatory locus might exhibit altered regulatory behavior.

Single-colony isolates of each of the mutants were grown in glucose medium, washed, and plated on G6P as sole carbon source. These plates were incubated at either 30 or 42 C. The numbers of uhp^+ revertants per 10^8 cells plated at either temperature are presented in Table 4.

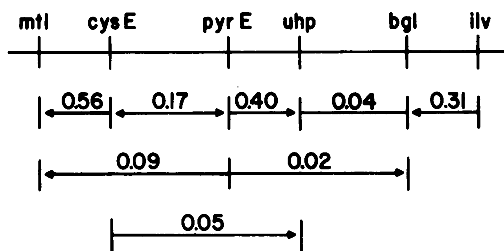

FIG. 2. *Cotransduction frequencies for markers in the vicinity of the uhp locus. Arrows point from selected marker to scored unselected donor marker; the double-headed arrow indicates the average of the cotransduction frequencies measured in both directions. The frequency for cotransduction of uhp and bgl was obtained from recombinants selected for pyr⁺. The values were calculated from the transductions described in Table 3 and Fig. 1.*

TABLE 4. *Properties of uhp⁺ revertants*

Allele no.	Revertants per 10^8 cells		Regulatory behavior		
	42 C	30 C	No. tested	No. inducible	No. constitutive
12	1.44	0.48	15	15	0
13	1.11	1.11	7	2	5
14	0.27	2.15	9	9	0
15	<0.1	2.27	10	9	1
17	1.03	0.45	9	5	4
18	0.39	8	—	—	—
19	0.36	0.36	2	2	0
20	3.44	0.55	10	2	8
21	9.65	4.76	7	4	3
22	0.35	1.18	—	—	—
23	0.88	2.46	6	6	0
24	1.85	2.31	8	5	3
25	1.51	0.82	11	6	5
26	11.4	10.7	3	0	3
27	1.46	0.73	13	9	4
28	9.71	6.07	7	7	0
29	0.47	<0.1	4	4	0

All of the mutations were able to revert, implying that they are all point mutations. Some of these revertants exhibited a temperature sensitivity for growth on G6P; these will be described in a subsequent communication.

A number of these revertants were tested for the regulatory behavior of the hexose phosphate transport system. Primarily, the revertants exhibited either normal inducibility or fully constitutive expression. The distribution of this regulatory behavior among the revertants studied is also shown in Table 4. There was no apparent correlation between the reversion frequency of a mutant allele and its ability to yield constitutive revertants. On the basis of the assumption mentioned above, it appears that 6 of 15 of the mutations may have affected the structural gene, whereas the remainder may have altered a regulatory locus. However, further work is necessary before any of these mutations can be assigned to a specific gene. It is of interest that such a large proportion of these mutants yielded revertants of constitutive phenotype.

DISCUSSION

This paper represents the initial report of a genetic analysis of the regulation of the hexose phosphate transport system. The primary interest in this project derives from the unusual regulatory behavior controlling this system, a phenomenon termed "exogenous induction" (17, 18). The inducing signal appears to be the presence of G6P on the outer surface of the membrane. Large pools of G6P can be established within the cell, but this pool apparently cannot serve directly as an inducer (7, 17). This compartmentalization of a regulatory effector is reminiscent of the findings of Sercarz and Gorini, which indicated that exogenous arginine was more effective for the repression of the arginine biosynthetic pathway than was arginine formed within the cell (14).

An initial analysis of the genetics of the hexose phosphate transport system has been provided by Kornberg and Smith (9). They showed that mutants lacking phosphoenolpyruvate-carboxylase were inhibited in their growth on acetate by the addition of carbohydrates, such as glucose or G6P. Mutants resistant to growth inhibition by G6P, but still sensitive to glucose, were defective in G6P transport. These mutations gave about 50% cotransduction with *pyrE*. We have extended this finding with the mapping of a large number of mutants in this system which were isolated by two different selection procedures. Further, we have defined the gene order in this region.

Mutants which produced the hexose phosphate transport system constitutively were also isolated in Kornberg's laboratory (4). These mutations apparently are alterations of the regulatory system. These mutants, which also cotransduced with *pyrE*, were isolated by selection for growth on fructose-1-phosphate. We have isolated similar mutants, and these will be described later.

However, we found that among the mutants defective in hexose phosphate transport were a number whose revertants exhibited altered regulation. This implies that the original defect in these mutants might have been in a regulatory gene, although further work is necessary for such an assignment. Our finding that these mutations mapped in the same region of the chromosome as the other uhp^- mutants implies that the regulatory locus is closely linked to the structural gene for this system. Experiments are in progress to provide the fine-structure mapping of this region, further characterization of revertants, and the dominance relationships of the mutant alleles.

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